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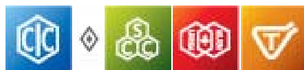

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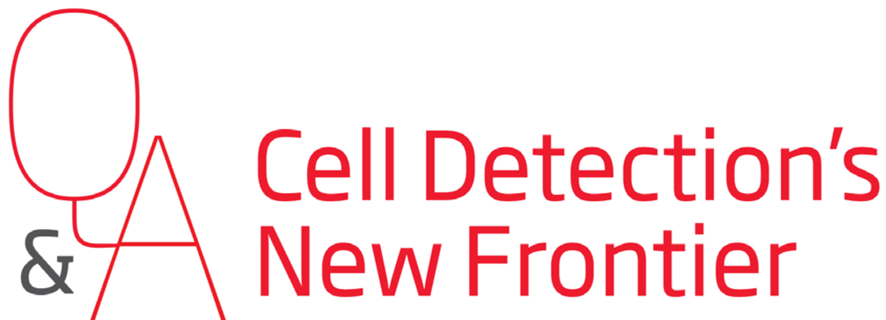
Mass Cytometry



Mass cytometry — an amalgam of atomic mass spectrometry and flow cytometry — raises the bar for high-resolution detection and quantitative assay in the field of cell biology.

By ***Tyler Irving***

Atomic mass spectrometry blasts molecules into tiny bits and examines the pieces. Flow cytometry gently probes cells with sophisticated antibodies. University of Toronto chemistry professor Scott Tanner, CEO of DVS Sciences, has merged these seemingly disparate areas to create "mass cytometry," which has the potential to revolutionize cancer research and drug discovery.

ACCN: What are the limitations of flow cytometry today?

ST: Flow cytometry is the current state-of-the-art method for looking at the heterogeneity of cell populations. If you have a complex sample and you need to identify each individual cell in that population, flow cytometry is the way to do it. Flow cytometry operates by probing a cell with an antibody or some affinity reagent, to which a fluorophore is appended. Then, the cells flow one at a time through a laser excitation. You look at the forward scatter and side scatter, which tell you about cell size and granularity, and then you look at the emission of the fluorophores to get the presence and quantity of the antigens that you're probing with the antibodies.

The limitations are tied to the breadth of the emissions spectra. When you excite a fluorophore with a laser it will emit a bunch of photons with a fairly broad distribution of wavelengths. And if you try to measure more than four of those fluorophores, then the emissions begin to overlap. If you have a very intense signal in one channel and a very weak signal in another channel, then you'll have a lot of spillover into the weaker channel. So you have to compensate for that; you have to do an assay that measures what the overlap is into each channel.

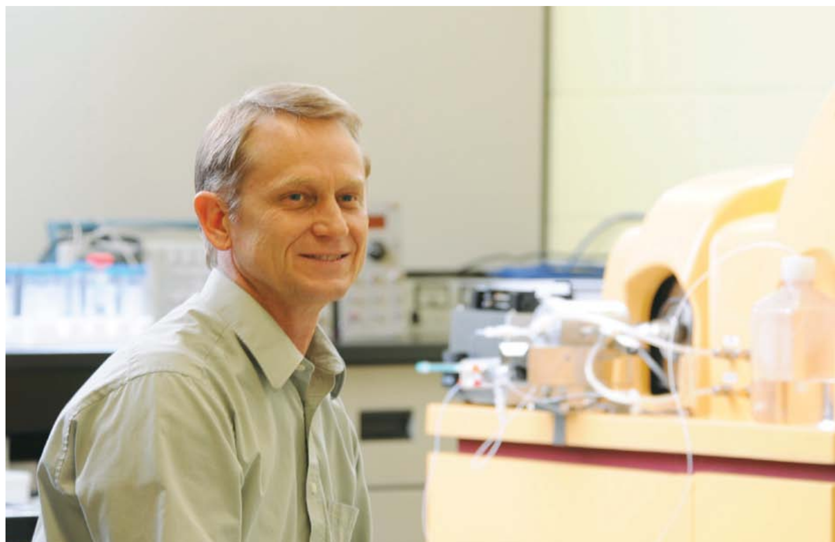
ACCN: How many different fluorophores can you keep track of at once?

ST: Most researchers can do four. There are some who can do eight, some superb people who can do 12, and a few champions can do as many as 17. The issue is that when you get up to a number of parameters like that, the selection of the fluorophores is an onerous task, because you have to know what you're going to be seeing at what intensity level. Then you have to choose your panel so that you put your bright fluorophores on your weak signals, and your weak fluorophores on your strong signals, and then try to label your samples so that all the signals are more or less the same height to minimize the amount of spillover. It could take six months to do a 12-colour panel selection.

ACCN: How did you become convinced that atomic mass spectrometry could overcome those limitations?

ST: Atomic mass spectrometry has a 30-year history of being state of the art for measurement of the atomic composition of matter, such as the amount of beryllium in a nuclear fuel rod or the amount of arsenic in well water. In the atomic mass spectrometer, you have a high temperature (7000 °K) flowing plasma. When you put a sample into that stream, it reduces your sample to the atomic state and then it ionizes those atoms. Then we sample those ions. Atomic mass spectrometry has been very good at resolving individual masses, because it is used for isotope analysis.

The 'aha moment' was recognizing that one can append a specific type of atom to an antibody or an affinity product. Then you can use the affinity product to provide the specificity and the atom becomes the probe that is measured. You can look at the atomic composition of the cell, but what you're really looking at is the atomic composition of the probes, which are surrogates for the antigens that you're measuring. So really it was just transferring the concepts of a fluorescent assay into the atomic mass spectrometry world, where you get the advantages of high-resolution detection, quantitative assay, enormous dynamic range — all the kinds of things you want in an analytical platform.



Scott Tanner is the inventor of the CyTOF, an analytical instrument which applies atomic mass spectrometry techniques to the study of cell populations

ACCN: What elements did you use? Was it easy to create these new element-tagged antibodies?

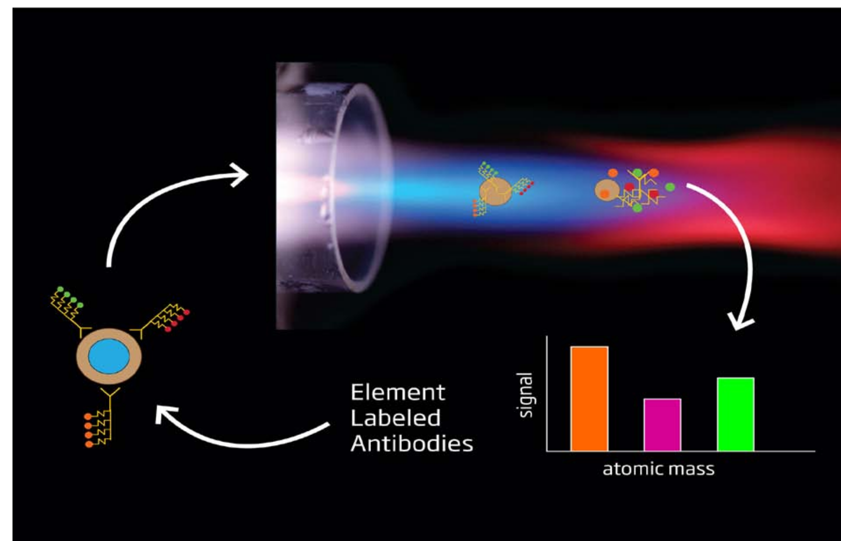
ST: It's always easy in hindsight. The nice thing about the periodic table is that it is periodic, there are 13 lanthanide elements with 37 different stable isotopes and the chemistry of the lanthanides is similar. If you know how to bind one lanthanide atom, then you know how to bind each of them. As soon as I have one, say for holmium, then the same probe will work for terbium or thulium or ytterbium. So one construct automatically produces 37 different probes. We always thought that 37 was a pretty good start and that people would be happy with that, but as soon as you give them that they ask, "where are the next 30?" So the next 30 will be the noble metals. There's another 30 noble metals that are available as enriched isotopes, and they have a different chelator molecule. So that will give you a 60 or 65 parameter potential.

ACCN: Can you give some examples of applications where you would need to measure this many different parameters?

ST: My U of T research group works within the cancer stem cell community. The application is to identify the disease early, before it's turned into a blast stage, so that the therapies are less aggressive. To identify the cancer stem cell, you have to be able to identify a biomarker signature that is unique to that cell and not any other cell. Statistically, because there's only one cancer stem cell in a million cells, you need to have something like 20, 30 or 40 probes that give you a multivariate signature that is distinctive for that cancer stem cell. The same thing would be true if you're looking at circulating tumour cells; the signature for that rare cell gets hidden in the signature for all the other cells if you do a bulk analysis, so you need to do them individually.

The other one would be in drug discovery. Say you want to look at the effect of a particular drug candidate on the phosphorylation pathways that occur in a single cell. The conventional way is to look at the target and to look at a metabolite of that target, so you see one pathway within the cell. What we allow you to do is measure many of those simultaneously. We just had a paper published in *Science* that had 18 phosphorylated proteins being measured simultaneously in response to each drug candidate. It's looking at a bone marrow sample, which is a very complex sample. Additionally, it measured 13 cell surface probes to distinguish the dendritic cells from the stem cells, T-cells and B-cells, and then we can look at each one of those populations in 18 different dimensions of phosphorylation against each drug candidate. It gives an immense amount of data. So that allows you to look not only at the pathways you're targeting, but also parallel pathways that could also be stimulated or suppressed, and it gives you much more information about what the impact of this drug is. It allows the drug developer to fail early and fail often, which is the mantra for accelerating drug discovery cost-effectively.

ACCN: You first developed these element-tagged antibodies 10 years ago, why are they only starting to receive widespread attention now?



The CyTOF approach uses antibodies tagged with elements that are uncommon in biological samples (such as lanthanides),which bind to biomarkers on thesurface of cells. The construct is then fed into a high-temperature plasma, where it is atomized and ionized. By counting the ions of the rare element, information about the presence and number of the desired biomarker is obtained.

ST: We developed the concept of element-tagged immunoassays back in 2000, and showed its use in bulk analysis using conventional inductively coupled plasma mass spectrometry (ICP-MS); there are several people around the world who are doing that now. Afterward, we got talking with John Dick, senior scientist at the Ontario Institute for Cancer Research, who was the first to identify the stem cell basis of leukemia. He pointed out to us that we really wanted to look at the heterogeneity of cell samples, and therefore we wanted to emulate flow cytometry. At that point we had moved into the U of T and were generously funded by federal and provincial bodies, so could do the development under the radar scope of the biological community. Both the tagging constructs and the instrument that's going to

read those were at a commercial level by 2009. So that's when we told the world about it. There's a huge buzz happening now, largely because of the promotions coming out of Garry Nolan's group at Stanford University. Garry was one of the early leaders in flow cytometry and he cottoned onto the idea instantly. We've got six instruments in the field at the moment: there's one in Toronto, two at Stanford, one at the National Institutes of Health , one in Taiwan and one in Japan.

ACCN: How much does this cost?

ST: The cost is $600,000 for the instrument and today's tagging constructs are $400 to label 100 micrograms of antibody. In the old days of fluorescence, 30 years ago, people would buy fluorophore labelling kits that they would append to their own antibodies. But in the past 30 years, companies have started selling catalogues of pre-conjugated antibodies. So our next step is to provide the same solution. Today we sell kits and the customer conjugates those antibodies. DVS Sciences is opening a new office in California where we're going to do the conjugation and sell conjugated antibodies from a catalogue.

ACCN: Aren't lanthanides quite rare and expensive?

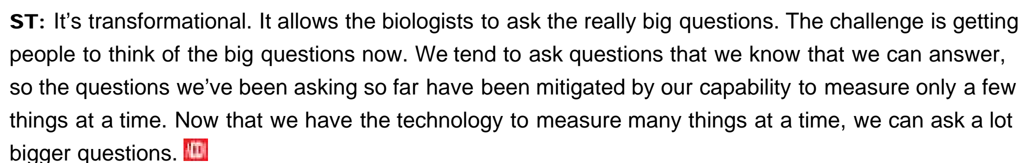
ST: The answer is yes; the caveat is that the detector is incredibly sensitive, so you don't need to use much of them. The stable isotopes are available commercially, there are a number of companies that sell them and there's a catalogue that you get. A certain number of them are available off the shelf, and a certain number can be produced for you in a certain period of time, so it's a matter of planning: you order them, you schedule it out, they arrive in. An order of a few milligrams of a stable isotope actually provides many tagging constructs.

ACCN: What has it been like going from being a researcher to being a CEO?

ST: It's been an interesting go. When you're a small venture, it's not that different from being a professor; it's a matter of managing a small budget and trying not to go broke. We've recently taken venture support for the company; we've really gone to a big scale in the last few months. It's a totally new growth opportunity for me, I had a lot of years experience in the business community, but more on the technical side of the business than on the management side of the business. So once again we do it exactly like I did my technology, I collaborate with people that give me great business advice.

ACCN: What will be the effect of this technology on cancer research and drug discovery?

ST: It's transformational. It allows the biologists to ask the really big questions. The challenge is getting people to think of the big questions now. We tend to ask questions that we know that we can answer, so the questions we've been asking so far have been mitigated by our capability to measure only a few things at a time. Now that we have the technology to measure many things at a time, we can ask a lot bigger questions.



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